

Serial No. 09/204,865

EXHIBIT A

1. (Twice Amended) A flow-through device for capturing a target nucleic acid comprising a three-dimensional porous substrate having immobilized thereon about 6×10^{-17} to 6×10^{-16} nmol/nm² of a capture polynucleotide which is capable of hybridizing to the target nucleic acid, and wherein said porous substrate is about 1 mm to 20 mm thick.
2. (Twice Amended) The flow-through device of Claim 58, 59 or 60 in which said porous substrate is about 1 mm to 20 mm thick.
3. (Twice Amended) The flow-through device of Claim 1, 58 or 59 in which said porous substrate has an average pore size of about 1 μ m to about 250 μ m.
4. (Twice Amended) The flow-through device of Claim 58, 59 or 60 in which said porous substrate has immobilized thereon about 2×10^{-19} to 2×10^{-15} nmol/nm² of said capture polynucleotide.
5. (Amended) The flow-through device of Claim 1, 58, 59 or 60 in which said capture polynucleotide is covalently attached to the porous substrate.
6. (Amended) The flow-through device of Claim 1, 58, 59 or 60 in which said capture polynucleotide is covalently attached to the porous substrate *via* a phosphodiester, phosphorothioate or phosphoramidate linkage.
7. (Amended) The flow-through device of Claim 1, 58, 59 or 60 in which said capture polynucleotide is covalently attached to the porous substrate *via* a carboxamide linkage.
8. (Twice Amended) The flow-through device of Claim 1, 58, 59 or 60 in which said capture polynucleotide is covalently attached to the porous substrate *via* a linker.

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9. (Twice Amended) The flow-through device of Claim 1, 59 or 60 in which said porous substrate is composed of glass or a polymeric material selected from the group consisting of polyethylene, polystyrene, polycarbonate and polypropylene.

10. (Thrice Amended) The flow-through device of Claim 1, 58, 59 or 60 in which said porous substrate is composed of high density or ultra-high molecular weight polyethylene.

11. (Twice Amended) The flow-through device of Claim 1, 58 or 60 in which said porous substrate has a void volume in the range of about 1 $\mu\text{l}/\text{cm}^2$ to about 100 $\mu\text{l}/\text{cm}^2$.

13. (Twice Amended) The flow-through device of Claim 1, 58 or 59 in which the porous substrate has a porosity in the range of about 25 to 80%.

14. (Twice Amended) The flow-through device of Claim 1, 58, 59 or 60 in which the capture polynucleotide is covalently immobilized on the porous substrate via its 5'- or 3'-terminal residue.

15. The flow-through device of Claim 14 further including a linker disposed between the porous substrate and the capture polynucleotide.

21. (Twice Amended) The flow-through device according to Claim 1, 58, 59 or 60 further comprising a housing in which the three-dimensional porous substrate is disposed.

22. (Amended) The flow-through device of Claim 21, in which said housing is selected from the group consisting of a syringe barrel, a pipette, a disposable pipette tip, a chromatography column, a spin column, a microchannel, a capillary and a multi-well plate.

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23. (Amended) A method of capturing a target nucleic acid from a sample, said method comprising flowing a sample containing or suspected of containing a target nucleic acid through a flow-through device according to Claim 1 under conditions wherein said target nucleic acid and capture polynucleotide hybridize to one another to form a hybridized complex, thereby capturing the target nucleic acid.

24. (Twice Amended) The method of Claim 23, 62, 63 or 64 in which said target nucleic acid is applied to said flow-through device under conditions of high stringency.

25. (Twice Amended) The method of Claim 23, 62, 63 or 64 in which said target nucleic acid is applied to said flow-through device under conditions of low stringency.

26. (Twice Amended) The method of Claim 23, 62, 63 or 64 in which said target nucleic acid is applied to the flow-through device under conditions wherein it hybridizes with said capture polynucleotide in less than one minute.

27. (Twice Amended) The method of Claim 23, 62, 63 or 64 in which said porous substrate of said flow-through device has an average pore size of about 1 μm to about 250 μm .

28. (Twice Amended) The method of Claim 62, 63 or 64 in which the density or surface concentration of said capture polynucleotide is about 2×10^{-19} to 2×10^{-15} nmol/nm².

29. (Amended) The method of Claim 23, 62, 63 or 64 in which said capture polynucleotide is covalently attached to the porous substrate of the flow-through device.

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30. (Amended) The method of Claim 23, 62, 63 or 64 in which said capture polynucleotide is covalently attached to the porous substrate of the flow-through device *via* a phosphodiester, phosphorothioate or phosphoramidate linkage.

31. (Amended) The method of Claim 23, 62, 63 or 64 in which said capture polynucleotide is covalently attached to the porous substrate of the flow-through device *via* a carboxamide linkage.

32. (Twice Amended) The method of Claim 23, 62, 63 or 64 in which said capture polynucleotide is covalently attached to the porous substrate of the flow-through device *via* a linker.

33. (Twice Amended) The method of Claim 23, 63 or 64 in which said porous substrate of said flow-through device is composed of glass or a polymeric material selected from the group consisting of polyethylene, polystyrene, polycarbonate and polypropylene.

34. (Twice Amended) The method of Claim 23, 62, 63 or 64 in which said porous substrate of said flow-through device is composed of high density or ultra-high molecular weight polyethylene.

35. (Twice Amended) The method of Claim 23, 62, 63 or 64 in which said porous substrate of said flow-through device has a void volume in the range of 0.1 $\mu\text{l}/\text{cm}^2$ to about 100 $\mu\text{l}/\text{cm}^2$.

36. (Twice Amended) The method of Claim 23, 62, 63 or 64 which further includes the step of washing said hybridized complex under conditions of moderate or high stringency.

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40. (Twice Amended) A method of determining whether a sample contains a target nucleic acid, said method comprising the steps of:

- (a) flowing a sample suspected of containing a target nucleic acid through a flow-through device according to Claim 1, 58, 59 or 60 under conditions wherein the target nucleic acid and capture polynucleotide hybridize; and
- (b) detecting the presence of hybrids, wherein a positive detection indicates the presence of the target nucleic acid in the sample.

41. The method of Claim 40, in which said target nucleic acid bears a reporter moiety and hybrids are detected by detecting the presence of said reporter moiety.

44. (Twice Amended) A kit for capturing a target nucleic acid of interest from a sample, comprising:

- a) a flow-through device according to Claim 1, 58, 59 or 60; and
- b) a housing into which the flow-through device can be disposed.

50. (Twice Amended) A kit for capturing a target nucleic acid from a sample comprising:

- a) a three-dimensional porous substrate having an average pore size of about 10 μm to about 100 μm and a porosity in the range of 25% to 80%; and
- b) a capture polynucleotide capable of being covalently attached to the porous substrate.

51. The kit of Claim 50 further including a linker capable of being covalently attached to the porous substrate and the capture polynucleotide.

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52. (Twice Amended) A kit for capturing a target nucleic acid from a sample comprising:

- a) a three-dimensional porous substrate having an average pore size of about 10 μm to about 100 μm and a porosity in the range of 25% to 80%; and
- b) means for generating a capture polynucleotide which is capable of hybridizing to the target nucleic acid and which is capable of being covalently attached to the porous substrate.

58. (Amended) A flow-through device for capturing a target nucleic acid comprising a three-dimensional porous substrate having immobilized thereon a capture polynucleotide which is capable of hybridizing to the target nucleic acid, and wherein said porous substrate is composed of a polymeric material selected from the group consisting of polyethylene, polystyrene, polycarbonate and polypropylene.

59. (Amended) A flow-through device for capturing a target nucleic acid, comprising a three-dimensional porous substrate having substantially irreversibly immobilized thereon a capture polynucleotide which is capable of hybridizing to the target nucleic acid, and wherein said porous substrate, prior to immobilization of the capture polynucleotide, is activated by plasma activation.

60. (Amended) A flow-through device for capturing a target nucleic acid, comprising a three-dimensional porous substrate having an average pore size of about 10 μm to about 100 μm and a porosity in the range of about 25 to 80% and having immobilized thereon a capture polynucleotide capable of hybridizing to the target nucleic acid.

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62. A method of capturing a target nucleic acid from a sample, said method comprising flowing a sample containing or suspected of containing a target nucleic acid through a flow-through device according to Claim 58 under conditions wherein said target nucleic acid and capture polynucleotide hybridize to one another to form a hybridized complex, thereby capturing the target nucleic acid.

63. A method of capturing a target nucleic acid from a sample, said method comprising flowing a sample containing or suspected of containing a target nucleic acid through a flow-through device according to Claim 59 under conditions wherein said target nucleic acid and capture polynucleotide hybridize to one another to form a hybridized complex, thereby capturing the target nucleic acid.

64. A method of capturing a target nucleic acid from a sample, said method comprising flowing a sample containing or suspected of containing a target nucleic acid through a flow-through device according to Claim 60 under conditions wherein said target nucleic acid and capture polynucleotide hybridize to one another to form a hybridized complex, thereby capturing the target nucleic acid.

65. The kit of Claim 50 or 51 in which the porous substrate is activated with about 6×10^{-17} to 9×10^{-15} nmol/nm² of a reactive group.

66. The kit of Claim 50 or 51 in which the porous substrate is composed of glass or a polymeric material selected from the group consisting of polyethylene, polystyrene, polycarbonate and polypropylene.

67. (Amended) The kit of Claim 66 in which the porous substrate is composed of high density or ultra-high molecular weight polyethylene.

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